临床研究

长方案添加黄体生成素对体外受精-胚胎移植结局的影响

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摘要：目的 比较在不同人群中添加黄体生成素在长方案超排卵过程中的临床结局。**方法** 选择2010年6月~2015年12月在广州市妇女儿童医疗中心生殖医学中心进行体外受精-胚胎移植助孕的671个周期，均使用自然周期黄体期长方案促排卵，按促排卵从启动日是否添加重组黄体生成素(r-LH)分为A组(单用卵泡刺激素FSH)和B组(FSH+r-LH)，再根据年龄是否小于35岁和启动日LH是否小于1.0 U/L分成4个亚组，分别比较不同亚组中是否添加LH对临床妊娠率和种植率的影响。**结果** 两组患者在年龄、体质量指数、基础FSH、基础LH、基础雌二醇(E2)、促性腺激素剂量及天数、扳机日LH、扳机日E2、扳机日孕酮、获卵数、受精率、每卵子可用胚胎率及优胚率上均相似，差异无统计学意义，仅在扳机日内膜厚度上差异有统计学意义；亚组分析显示：对于年龄<35岁且启动日LH<1.0 U/L及年龄≥35岁且启动日LH≥1.0 U/L的患者，单用FSH组和FSH+r-LH组在临床妊娠率和种植率上均相似，差异无统计学意义；对于年龄<35岁且启动日LH≥1.0 U/L的患者，FSH+r-LH组临床妊娠率60%，低于单用FSH组的临床妊娠率79.55%，差异有统计学意义($P<0.05$)；对于年龄≥35岁且启动日LH<1.0 IU/L的患者，FSH+r-LH组的种植率为44.74%，高于单用FSH组的种植率24.74%，差异有统计学意义($P<0.05$)。**结论** 在长方案中，与单用FSH相比，添加LH并不提高获卵数、受精率及每卵子优质胚胎率；对年龄<35岁即使降调节后LH水平低或年龄≥35岁但降调后LH≥1.0 U/L者添加LH并不能使患者明显获益，对于年龄≥35岁且降调后LH<1.0 U/L的患者添加LH可能改善临床妊娠率和种植率，而对于年龄<35岁且启动日≥LH1.0 U/L的患者应慎重选择添加外源性LH。

关键词：黄体生成素；长方案；临床妊娠率；种植率

Effects of luteinizing hormone supplementation on outcomes of *in vitro* fertilization and embryo transfer in patients undergoing GnRH-agonist long protocol

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Abstract: Objective To compare the clinical outcomes of *in vitro* fertilization and embryo transfer (IVF-ET) among patients in different conditions receiving luteinizing hormone supplementation in GnRH-agonist long protocol. **Methods** Between June, 2010 and December, 2015, 671 IVF-ET cycles with GnRH-agonist long protocol were performed at our center. These cycles were divided into group A with FSH only and group B with FSH and recombinant luteinizing hormone (r-LH) supplementation, and each group was divided into 4 subgroups according to age (<35 or ⩾35) and the LH level on the initial day (<1.0 U/L or ⩾1.0 U/L). The effects of LH supplementation on the clinical pregnancy rate and implantation rate were compared among different subgroups. **Results** No statistical significances were found between groups A and B in age, body mass index (BMI), basal FSH, basal LH, basal E2, Gn dosage, Gn day, LH on HCG day, E2 on HCG day, P on HCG day, number of oocytes, fertilization rate, available embryo rate or good quality embryo rate per oocyte, but the endometrium thickness on HCG day differed significantly between the two groups. In women below 35 years of age with a LH level on HCG day over 1.0 U/L, r-LH supplementation resulted in a clinical pregnancy rate of 60%, significantly lower than the rate of 79.55% in women without r-LH supplementation ($P<0.05$). In women over 35 years with a LH level below 1.0 U/L, r-LH supplementation resulted in an implantation rate of 44.74%, as compared with 24.74% in women without r-LH supplementation ($P<0.05$). **Conclusion** In the long protocol, LH supplementation does not improve the oocyte number, fertilization rate, or good quality embryo per oocyte, and does not bring benefits to women below 35 years with a low LH level (<1.0 U/L) or those over 35 years with normal LH level (⩾1.0U/L) after GnRH-agonist administration. But for women over 35 years with low LH levels, r-LH supplementation may improve the clinical pregnancy rate and implantation rate of IVF-ET cycles.

Keywords: luteinizing hormone; GnRH-agonist long protocol; clinical pregnancy rate; implantation rate

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"两细胞两促性腺激素"学说与近期基础研究都表明：腺垂体分泌的糖蛋白卵泡刺激素(FSH)和黄体生成素(LH)在自然周期卵泡发育过程中发挥着决定性的作用[1-2]。在长方案控制性促排卵中，为防止提前排卵，使用促性腺激素释放激素的激动剂抑制体内LH水平，低

LH水平对卵泡发育成熟及妊娠结局的影响一直存在争议，有学者认为，对多数正常反应者，内源性LH已能满足卵泡发育需要，不需要额外添加外源性LH，也有研究认为外源性补充LH能改善卵子和胚胎质量，提高妊娠结局[3-6]。因此，非选择性地添加外源性LH能否改善临床妊娠结局尚待探索。近年来，越来越多研究认识到可能存在某些特殊人群需要添加适量LH以提高卵巢反应性及改善妊娠结局，目前讨论最多的两种影响因素为年龄及血清LH水平，本研究首次将以上两个重要因素结合分析，旨在探寻外源性补充LH的适合和受益人群，以进一步改善体外受精-胚胎移植的妊娠结局。

1 资料和方法

1.1 研究对象

选取2010年6月~2015年12月在广州市妇女儿童医疗中心生殖医学中心行体外受精-胚胎移植助孕的671个周期资料进行回顾性分析。入组标准：年龄22~43岁，月经周期规律，均使用自然周期黄体期长方案促排卵；不孕原因主要为女方输卵管因素、男方因素及不明原因不孕等；排除多囊卵巢综合征，高泌乳素血症等内分泌疾病、未处理的输卵管积液、子宫内膜息肉、宫腔粘连及子宫内膜异位症患者。

1.2 分组和观察指标

按在促排卵中是否添加LH分为A组（单用FSH）525例和B组（FSH+r-LH）146例，比较两组年龄、体质量指数（BMI）、基础FSH、基础LH、基础E2、促性腺激素（Gn）剂量、Gn天数、扳机日LH、扳机日E2、扳机日孕酮（P）、扳机日内膜厚度、获卵数、受精率、每卵子可用胚胎率及优胚率的差异；再根据年龄是否小于35岁和启动日LH是否小于1.0 U/L分成4个亚组，亚组1（年龄<35岁，LH<1.0 U/L）、亚组2（年龄<35岁，LH≥1.0 U/L）、亚组3（年龄≥35岁，LH<1.0 U/L）及亚组4（年龄≥35岁，LH≥1.0 U/L），A组中4个亚组分别为187、88、86及27例，B组中4个亚组分别为48、25、20及6例，比较不同亚组中是否添加LH对临床妊娠率和种植率的影响。

1.3 治疗方案及结果判断

所有患者均采用自然周期黄体中期长方案进行控制性促排卵，黄体中期使用达菲林1.3 mg降调，降调14 d后若血清FSH<5 U/L、LH<5 U/L、E2<180 pmol/L，且双侧卵巢卵泡径线<10 mm），使用重组卵泡刺激素（r-FSH，果纳芬）75~225 U启动，其后阴道超声及性激素监测卵泡发育情况，在促排卵过程中从启动日开始不添加或添加重组黄体生成素（r-LH，乐芮）75 U/d，B超监测如果2个卵泡经线≥18 mm或3个卵泡经线≥17 mm时，使用重组人绒毛膜促性腺激素（r-hCG，艾泽）250 μg扳机，36 h后取卵，常规体外受精或卵胞浆内单精子显微注射，观察并记录卵子受精和胚胎发育情况，取卵后第3天选择1~2枚胚胎移植，常规黄体支持，移植后14 d测定血清绒毛膜促性腺激素（β-hCG）水平，β-hCG阳性为生化妊娠，验孕2周后腹部B超下见妊娠囊及原始心管搏动确定临床妊娠。

1.4 统计学分析

应用SPSS 19.0统计学软件进行分析，2PN率、可用胚胎率、优胚率、临床妊娠率及种植率的比较采用卡方检验，其他结果均以均数±标准差表示，采用*t*检验，$P<0.05$为差异有统计学意义。

2 结果

2.1 两组患者基本情况和胚胎质量的比较

两组患者在年龄、体质量指数、基础FSH、基础LH、基础E2、Gn剂量、Gn天数、扳机日LH、扳机日E2、扳机日P、获卵数、受精率、每卵子可用胚胎率及优胚率上均相似，差异无统计学意义，两组患者仅在扳机日内膜厚度上差异有统计学意义（$P<0.05$，表1）。

2.2 添加LH对不同亚组的临床妊娠率和种植率的影响

对于年龄<35岁且启动日LH<1.0 U/L及年龄≥35岁且启动日LH≥1.0 U/L的患者，单用FSH组和FSH+r-LH组在临床妊娠率和种植率上均相似，差异无统计学意义（表2、3）；对于年龄<35岁且启动日LH≥1.0 U/L的患者，FSH+r-LH组临床妊娠率60%，低于单用FSH组的临床妊娠率79.55%，差异有统计学意义（$P<0.05$，表2）；对于年龄≥35岁且启动日LH<1.0U/L的患者，FSH+r-LH组的种植率为44.74%，高于单用FSH组的种植率24.74%，差异有统计学意义（$P<0.05$，表3）。

3 讨论

黄体期降调节后，对垂体过度抑制可能导致体内LH水平过低。研究表明，长方案中若单用FSH，可能出现卵泡发育减慢，卵子质量、受精率、临床妊娠率和种植率降低，流产率升高，外源性补充LH能改善卵子和胚胎质量，提高妊娠结局[4-6]。前瞻性研究也提示：外源性添加LH能减少促排卵药用量和天数，增加正常受精胚胎数[7]。但也有学者认为，对多数正常反应患者，经降调后体内内源性分泌的LH已能满足卵泡发育需要，并不需要额外添加外源性LH。本研究的结果也表明，添加LH组与单用FSH组在Gn剂量、获卵数、受精率、每卵子可用胚胎率及优胚率上均相似，因此，长方案中添加LH对于促排卵治疗的作用至今仍存在争议[8-9]。近年来，多数研究提出，可能在某些特殊人群需要添加适量LH以提高卵巢反应性及改善妊娠结局，目前讨论最多的两种影响因素分别为年龄及血清LH水平，本研究针对目前添加LH的主要争论点年龄是否超过35岁及降调后启动

表1 两组患者基本情况和胚胎质量的比较

Tab.1 Comparison of clinical data and embryo quality between group A and group B

Characteristics	A group (*n*=525)	B group (*n*=146)	*P*
Age (year)	31.99±4.16	31.62±3.95	NS
BMI (kg/m^2)	20.90±2.52	21.41±2.78	NS
Basal FSH (U/L)	5.61±1.39	5.72±1.41	NS
Basal LH (U/L)	3.73±2.05	3.77±2.19	NS
Basal E2 (pmol/L)	137.64±85.76	159.11±197.10	NS
Dose of gonadotropin (U)	1957.36±794.97	2016.03±845.12	NS
Gn day	11.47±1.93	11.60±2.57	NS
LH on HCG day (U/L)	1.08±1.16	1.03±0.74	NS
E2 on HCG day (pmol/L)	9402.10±4712.40	9482.61±4966.33	NS
P on HCG day (nmol/L)	1.73±1.02	2.00±2.09	NS
Endometrium thickness on HCG day (mm)	11.26±2.70	12.18±2.35	0.026
Number of oocytes retrieved (*n*)	10.86±5.68	8.84±4.97	NS
2PN rate	66.00%	66.92%	NS
Embryos rate (per oocytes)	46.71%	46.79%	NS
High grade embryos rate (per oocytes)	17.48%	17.97%	NS

表2 亚组临床妊娠率的比较

Tab.2 Comparison of clinical pregnancy rate in the subgroups

Clinical pregnancy rate	LH on day of Gn<1.0 U/L			LH on day of Gn≥1.0 U/L		
	FSH only	FSH+r-LH	*P*	FSH only	FSH+r-LH	*P*
<35 year	52.94%	54.17%	NS	79.55%	60.00%	0.002
≥35 year	41.86%	60.00%	NS	59.26%	50.00%	NS

表3 亚组种植率的比较

Tab.3 Comparison of implantation rate in the subgroups

Implantation rate	LH on day of Gn<1.0 U/L			LH on day of Gn≥1.0 U/L		
	FSH only	FSH+r-LH	*P*	FSH only	FSH+r-LH	*P*
<35 year	36.49%	37.50%	NS	56.47%	45.45%	NS
≥35 year	24.74%	44.74%	0.02	33.90%	33.33%	NS

日LH水平是否小于1.0 U/L细分亚组分别进行研究，对不添加和添加r-LH的各项实验室及临床指标进行比较，旨在探寻外源性LH补充的适合和受益人群。

（1）高龄女性：尤其是超过35岁的女性，卵泡上颗粒细胞功能性LH受体数量和LH生物活性随年龄增长逐渐下降，雄激素分泌减少，添加LH可能获益[9-10]。已有多项随机对照试验提示对≥35岁患者添加外源性r-LH的好处。Matorras等[5]发现，对35~39岁患者添加r-LH获得更高的种植率和活产率。在FSH促排卵方案中添加重组LH，可以使卵巢低反应患者的临床妊娠率提高30%[11]。2011年有关超排卵周期中r-LH补充治疗的亚洲共识建议：年龄是女性缺乏LH生物活性的重要标志，建议>35岁的女性第6天起每日添加75U r-LH[12]。然而，也有研究得出不同结论：超过35岁女性添加LH对其继续妊娠率并无提高[13]，因此，本文分组的条件之一为年龄是否超过35岁。

(2)降调后LH水平低下者:2002年,Balasch与Fabregues[14]提出"LH阈值",即维持卵泡正常发育所需的最低LH水平,低于此阈值可能影响卵泡发育和成熟。前瞻性研究也表明:对降调节后LH明显抑制的患者添加LH有利于卵母细胞成熟和受精[15]。Kol等[16]也认为内源性LH变化是外源性补充LH的基础。然而,目前的研究并未发现内源性LH水平和妊娠率之间存在相关性[17],长方案降调后LH的水平在患者中的变异性较大[18],促排卵过程中血清LH水平的测定对于妊娠结局并无预测价值[19-20]。LH值低于何阈值才需要外源性添加,目前尚不明确。Fleming等[21]发现当卵泡中期LH<1.0 U/L时,E2水平降低,妊娠率下降。国内也有一些学者认为长方案垂体降调节后血清LH≤1.0 U/L时,HCG日血E2水平下降,受精率、优质胚胎率和临床妊娠率下降,流产率增加,因此,本文分组的另一条件为降调后LH是否小于1.0 U/L。最新的一项多中心随机对照试验结果显示:长方案中,启动后第6天血清LH水平比启动日下降超过50%以上者每日添加75 U乐芮,并不能够改善临床妊娠率和活产率[22]。同时,"LH窗"理论也认为,LH浓度过高反而可能导致卵子受损[23]。当促排卵中期LH>1.5 U/L时,添加LH与不添加组相比,获卵数增加,但卵子质量和临床妊娠率明显下降[24]。对正常人群常规加用LH并不改善助孕结局,有研究认为降调后早-中卵泡期LH的升降对结局无影响[25],非选择性加用LH可能反而不利于妊娠结局,供卵者降调后LH≥1.0 U/L者,促排第5天开始每日添加HMG75U,优质胚胎数和种植率均明显下降[26],这与本研究结果是一致的,对于年龄<35岁且启动日LH≥1.0 U/L的患者,添加外源性LH反而可能对结局有不利影响,因此临床上应该有针对性地选择性添加LH。

本研究首次将以上两个重要因素结合分析,结果表明:对于年龄≥35岁的患者,只有当LH<1.0 U/L时,添加LH才能改善妊娠结局;反之,当启动日LH<1.0 U/L时,若患者是年龄<35岁的正常反应者,添加外源性LH并不能改善结局,即当两个可能的不利因素都满足时,才需要外源性补充LH以改善胚胎利用率及妊娠结局。

本研究局限性:仅针对非选择性人群进行回顾性分析,并未对添加LH的时机进行分组讨论,还需加大样本量、设计前瞻性随机对照研究进一步对需要添加LH的指征、人群、时机和药物种类剂量等进行详细探讨。

综上所述,本文认为在长方案中,与单用FSH相比,添加LH并不能提高获卵数、受精率及每卵子优质胚胎率;对于年龄小于35岁即使降调节后LH水平低或年龄超过35岁但降调后LH高于1.0 U/L者添加LH并不能使患者明显获益,对于年龄超过35岁且降调后LH低于1.0 U/L的患者添加r-LH可能改善临床妊娠率和种植率,而对于年龄小于35岁且启动日超过LH1.0 U/L的患者应慎重选择添加外源性LH。

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